

AMENDMENTS TO THE SPECIFICATION

Please replace paragraph [0014] with the following rewritten paragraph:

The isolated promoter was found to direct high-level seed-associated gene expression when operably ~~lined~~linked to heterologous coding sequences. As used herein, the terms "isolated PRU promoter" and "chPRU promoter" refer to a nucleic acid comprising the sequence provided in SEQ ID NO:1 or the reverse complement thereof, SEQ ID NO:6. The term also encompasses fragments and derivatives thereof that retain seed-associated promoter activity as discussed in more detail below. As used herein, the term "seed-associated promoter" refers to a promoter that directs RNA synthesis at higher levels in seeds than in other cells and tissues. A "seed-specific" promoter is a seed-associated promoter that directs RNA synthesis essentially only in the seed. But, under certain conditions and using particular detection methods, very low levels of expression in tissue other than seed may be detected from a seed-specific promoter.

Please replace paragraph [0018] with the following rewritten paragraph:

In one embodiment, derivatives include insertions, deletions (including 5' and/or 3' truncations) and substitutions of one or more nucleotides. Such derivatives may be naturally occurring (e.g., polymorphic sequences) or may be synthetic (including variants of the disclosed sequences that result from site-directed or random mutagenesis) and may be obtained using methods known to those skilled in the art. Methods by which one may empirically determine whether a candidate derivative sequence is sufficiently homologous to the isolated PRU promoter to —direct seed-associated gene expression are well known in the art and are described herein.

Please replace paragraph [0024] with the following rewritten paragraph:

Derivative sequences may be identified by their ability to hybridize to an isolated PRU promoter under stringent hybridization conditions. The stringency of hybridization can be controlled by temperature, ionic strength, pH, and the presence of denaturing agents such as formamide during hybridization and washing. Conditions routinely used are set out in readily available procedure texts (e.g., Current Protocol in Molecular Biology, Vol. 1, Chap. 2.10, John Wiley & Sons, Publishers (1994); Sambrook *et al.*, Molecular Cloning, Cold Spring Harbor (1989)). In some embodiments, a nucleic acid molecule of the invention is capable of

hybridizing to a nucleic acid molecule containing the nucleotide sequence of SEQ ID NO:1 or the complement or thereof under stringent hybridization conditions that comprise: prehybridization of filters containing nucleic acid for 8 hours to overnight at 65° C in a solution comprising 6X single strength citrate (SSC) (1X SSC is 0.15 M NaCl, 0.015 M Na citrate; pH 7.0), 5X Denhardt's solution, 0.05% sodium pyrophosphate and 100 µg/ml herring sperm DNA; hybridization for 18-20 hours at 65° C in a solution containing 6X SSC, 1X Denhardt's solution, 100 µg/ml yeast tRNA and 0.05% sodium pyrophosphate; and washing of filters at 65° C for 1 h in a solution containing 0.2X SSC and 0.1% SDS (sodium dodecyl sulfate). In other embodiments, moderately stringent hybridization conditions are used that comprise: pretreatment of filters containing nucleic acid for 6 h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll™, 1% BSA, and 500 µg/ml denatured salmon sperm DNA; hybridization for 18-20 h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll™, 0.2% BSA, 100 µg/ml salmon sperm DNA, and 10% (wt/vol) dextran sulfate; followed by washing twice for 1 hour at 55° C in a solution containing 2X SSC and 0.1% SDS. Alternatively, low stringency conditions can be used that comprise: incubation for 8 hours to overnight at 37° C in a solution comprising 20% formamide, ~~5-5X~~ SSC, 50 mM sodium phosphate (pH 7.6), 5X Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured sheared salmon sperm DNA; hybridization in the same buffer for 18 to 20 hours; and washing of filters in ~~4-1X~~ SSC at about 37° C for 1 hour.

Please replace paragraph [0034] with the following rewritten paragraph:

The invention provides the first example of a bi-directional promoter that controls similar seed-associated expression in each orientation. The bi-directionality of the isolated PRU promoter provides specific advantages for the genetic engineering of plants. First, the feature facilitates the introduction of multiple genes into plants, which is often necessary for metabolic engineering and trait stacking. Use of the bi-directional promoter may further avoid gene silencing, which may be induced by the repeated use of a single promoter in a cell.

Please replace paragraph [0047] with the following rewritten paragraph:

An important aspect of the invention is the ability to limit the expression of a heterologous gene under control of a PRU promoter to the seed of a transgenic plant. Altering the primary metabolic pathways that control carbohydrate, lipid, and/or amino acid production may have detrimental consequences on plant performance or yield if these metabolic changes occur in all tissues of the plant. For example, the global elimination of polyunsaturated fatty acid production results in plants that are no longer photoautotrophic (McConn and Browse 1998, *Plant J.* 15:521-30); however, when such fatty acid alterations are confined to seed, they have had no effect on plant productivity (Mazur et al. 1999, *Science* 285:372-5). For other applications, a desired trait may depend on the localized toxicity of the gene product. For instance, expression of genes that are deleterious or toxic may ~~been~~be used to create seedless plant varieties.

Please replace paragraph [0050] with the following rewritten paragraph:

A PCR-accessible genomic library was constructed from DNA extracted from cherry leaves (*Prunus avium*). Oligonucleotide primers were designed ~~complementary~~complementary to the 5' sequence of the Pru1 prunin cDNA clone (GenBank accession X78119, gi|460805) from almond (pru1 PFa, SEQ ID NO:4; pru1 PFb, SEQ ID NO:5) and were used to amplify a genomic fragment of upstream sequence from a set of cherry (*Prunus avium*) promoter finder (PF) libraries. The amplification resulted in a 1.2kb fragment present in all of the promoter finder libraries, which was an interesting result since the products from each separate library are determined by the position of a unique restriction site.

Please replace paragraph [0052] with the following rewritten paragraph:

Furthermore, the primer sequence ~~complementary~~complementary to Pru1 was detected on both the 5' and 3' ends of the clone, which would explain the unusual result of the genomic DNA amplification. The results indicated that there are two homologous Prunin-like genes in the cherry genome that are in opposite orientations and separated by 1.2 kb of upstream sequence. A single EcoRI site occurs in the center of the sequence. It can also be inferred, due to the absence of any other amplification products from the PF libraries, these may be the only two copies of Prunin-like genes in the cherry genome.

Please replace paragraph [0061] with the following rewritten paragraph:

Fatty acid methyl ester (FAME) analysis. Fatty acid methyl esters were prepared from leaves and seeds of transformant lines carrying the three above-described constructs and ~~control~~control lines (both wild type Col-0 and *fad2* mutant). Quantitative determination of leaf and seed fatty acid composition was performed as follows. Either whole seeds or cut leaves were trans-esterified in 500 μ l 2.5% ~~H₂SO₄~~H₂SO₄ in MeOH for 3 hours at 80 degrees C, following the method of Browse et al. (Biochem J 235:25-31, 1986) with modifications. A known amount of heptadecanoic acid was included in the reaction as an internal standard. 750 μ l of water and 400 μ l of hexane were added to each vial, which was then shaken vigorously and allowed to phase separate. Reaction vials were loaded directly onto GC for analysis, and the upper hexane phase was sampled by the autosampler. Gas chromatography with Flame Ionization detection was used to separate and quantify the fatty ~~Acid~~acid methyl esters. AgilentTM 6890 Plus ~~GC's~~GCs were used for separation with Agilent InnnowaxTM columns (30m x 0.25mm ID, 250~~umum~~um film thickness). The carrier gas was ~~Hydrogen~~hydrogen at a constant flow of 2.5 ml/ minute. 1 ~~ul~~ μ l of sample was injected in splitless mode (inlet temperature 220 °C, Purge flow 15ml/min at 1 minute). The oven was programmed for an initial temperature of 105°C, Initial Time 0.5 minutes, followed by a ramp of 60°C per minute to 175°C, a 40°C /minute ramp to 260°C with a final hold time of 2 minutes. Detection was by Flame Ionization (Temperature 275°C, Fuel flow 30.0 ml/min, Oxidizer 400.0 ml/min). Instrument control and data collection and analysis was using the MillenniumTM Chromatography Management System (Version 3.2, Waters Corporation, Milford, MA). Integration and quantification was performed automatically by the MillenniumTM software.